

# A Case–Control Study of Maternal Polybrominated Diphenyl Ether (PBDE) Exposure and Cryptorchidism in Canadian Populations

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**BACKGROUND:** Polybrominated diphenyl ethers (PBDEs) are flame retardants found in North American household products during the past four decades. These chemicals leach out in dust as products age, exposing individuals daily through inhalation and ingestion. Animal studies suggest that PBDEs disrupt sex hormones and adversely affect development of the reproductive system.

**OBJECTIVES:** In the present study, we examined whether there is a link between maternal hair PBDE concentrations and the risk of cryptorchidism (undescended testes) in male infants; testis descent is known to be dependent on androgens.

**METHODS:** Full-term male infants were recruited through clinics in Montreal, Toronto, and London, Canada. Boys with cryptorchidism at 3–18 months of age ( $n = 137$ ) were identified by pediatric urologists and surgeons; similar-aged controls ( $n = 158$ ) had no genitourinary abnormalities as assessed by pediatricians. Eight BDE congeners (BDE-28, -47, -99, -100, -153, -154, -183, -209) were measured by GC-MS (gas chromatography–mass spectrometry) in maternal hair samples collected at the time of recruitment.

**RESULTS:** The  $\Sigma$  PBDE geometric mean for maternal hair was 45.35 pg/mg for controls and 50.27 pg/mg for cases; the concentrations of three BDEs (BDE-99, -100, and -154) were significantly higher in cases than controls in unadjusted models. In adjusted models, every 10-fold increase in the concentration of maternal hair BDE-99 [OR = 2.53 (95% CI: 1.29, 4.95)] or BDE-100 [OR = 2.45 (95% CI: 1.31, 4.56)] was associated with more than a doubling in the risk of cryptorchidism. BDE-154 [OR = 1.88 (95% CI: 1.08, 3.28)] was also significant.

**CONCLUSIONS:** Our results suggest that maternal exposure to BDE-99, -100, and -154 may be associated with abnormal migration of testes in the male fetus. This may be due to the anti-androgenic properties of the PBDEs. <https://doi.org/10.1289/EHP522>

## Introduction

Cryptorchidism is the failure of one or both testicles to descend into the scrotum during *in utero* development of the male fetus (Barteczko and Jacob 2000). This is one of the most common (1.8–9%) urogenital abnormalities observed in normal term male newborns (Virtanen and Toppari 2008). In brief, two stages are involved in testis migration (Barthold 2008). The first occurs between gestational weeks 8 and 15, when the testicles travel from an intra-abdominal perirenal position to the top of the inguinal ring. Late in the third trimester, they then migrate through the inguinal ring and into the scrotal sac. In certain cases, the testes do not undergo the final migration until after birth but by 3 months the majority will have descended, spontaneously reducing the number of cases that require surgery (orchidopexy) to reposition the testes within the scrotum (Kollin and Ritzén 2014). Orchidopexy is recommended between ages of 6 and 12 months

to decrease the risk of testicular torsion or trauma, improve fertility and decrease the risk of testicular neoplasm in adulthood.

Animal and clinical studies have demonstrated that normal migration of the testes is dependent on both genetic factors and the *in utero* hormonal environment (Barthold 2008; Barthold et al. 2015; Huang et al. 2012; Jensen et al. 2010; Virtanen and Toppari 2008). The trans-abdominal phase is linked to expression of two genes: one for the insulin-like peptide-3 (*INSL-3*) hormone produced by Leydig cells and a second for the *INSL-3* receptor, relaxin-family peptide receptor 2 (*RXFP2*). The second inguinal–scrotal phase is thought to be primarily dependent on androgens produced by the fetal Leydig cells and normal expression of the androgen receptor. Clinical reports have linked cryptorchidism with mutations in the *INSL-3*, *RXFP2*, or *Androgen Receptor* (*AR*) genes but only in a small number of cases (Bay et al. 2011; Feng et al. 2009; Ferlin et al. 2009). Thus, the etiology of most cases remains unknown. In a study that evaluated the risk contribution from genetic versus intrauterine environmental factors, Jensen et al. (Jensen et al. 2010) found a similar concordance rate in monozygotic and dizygotic twins, providing strong support for an important role of the intrauterine environment.

There is increasing evidence that maternal exposure to certain environmental chemicals may have endocrine disrupting activity at critical stages during testicular development and/or migration due to the ability of these compounds to cross the placenta and enter the fetal environment (Bay et al. 2011; Virtanen and Adamsson 2012). Such chemicals include flame retardants, organochlorine pesticides, fungicides, dioxins, bisphenol A, and phthalates, all of which exhibit estrogenic or anti-androgenic properties in *in vitro* assays (Balbuena et al. 2013; Christen et al. 2014; Hamers et al. 2006; Harju et al. 2007; Rosenmai et al. 2014; Rouiller-Fabre et al. 2015; Stoker et al. 2005; Yang

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et al. 2009) and have been linked to genitourinary malformations, including cryptorchidism, in animal studies (Auger et al. 2014; Chen et al. 2015; Christiansen et al. 2009; Christiansen et al. 2010; Christiansen et al. 2014; Emmen et al. 2000; van den Driesche et al. 2012; Welsh et al. 2008). However, evidence for their effects on cryptorchidism in humans remains controversial. Many studies have involved small cohorts and have shown possible, but not significant, associations (Bay et al. 2011; Chevalier et al. 2015; Cook et al. 2011; Jensen et al. 2015; Koskenniemi et al. 2015; Virtanen and Adamsson 2012). One small study (62 cases, 68 controls) of a Danish mother–infant cohort reported significant associations between breast milk levels of several polybrominated diphenyl ether (PBDE) flame retardants measured 1–3 months after birth with increased risk of cryptorchidism at birth (Main et al. 2007). In a parallel Finnish cohort, no associations were found, despite similar total PBDE levels in breast milk; however, the PBDE congener profile differed (Krysiak-Baltyn et al. 2012; Main et al. 2007).

Because of the contradictory reports in the literature, the goal of the present study was to reexamine the possible association of maternal PBDE exposure and increased risk of undescended testes in male infants. We chose a cohort where the cryptorchid cases were defined by direct observation during orchidopexy. Mother/child pairs were recruited 3–18 months after birth because testes may spontaneously descend during the first 3–6 postnatal months and surgery only occurs several months after diagnosis of cryptorchidism. Because prior studies examined testes descent at birth, we hypothesized that the previous inconsistent results might be due, in part, to case misclassification. Thus, this study was designed to have an unambiguous cohort of cryptorchid male infants.

## Methods and Materials

### Participants

A total of 374 mother and child pairs were recruited between the summer of 2011 and summer of 2014. The research study was approved by the ethics boards at the different recruitment centers and all participants signed a written informed consent. The cryptorchidism cases ( $n=200$ ) were recruited at Pediatric Urology and General Surgery clinics at the Montreal Children's Hospital ( $n=80$ ), the Hospital for Sick Children in Toronto ( $n=115$ ) and the London Health Sciences Centre in London, Ontario ( $n=5$ ) following diagnosis by pediatric urologists and surgeons. Diagnosis between centers followed accepted standards: all centers reviewed and agreed on the diagnostic criteria (Wein et al. 2016). Infants with retractile testes were excluded from the study. Controls ( $n=174$ ) were recruited through the Hospital for Sick Children helpline for questions about pregnancy and breastfeeding ( $n=51$ ) and at a Montreal community pediatric center ( $n=123$ ): pediatricians verified the lack of urogenital abnormalities. Mothers were eligible to participate if they were  $\geq 18$  years old, had sufficient hair to provide a sample, and had a child who was between the ages of 3 and 18 months, born full term ( $\geq 37$  weeks gestation) with normal weight ( $\geq 2,500$  g), diagnosed with or without cryptorchidism, and with no other genitourinary malformations or genetic syndromes. Participation rate was  $>95\%$ .

Mothers in both groups filled out a questionnaire with information pertaining to home and work environment, general medical history, reproductive history (including paternal and familial history of cryptorchidism), breastfeeding, diet, alcohol consumption, smoking, medication usage and maternal socio-demographics (age, birth place, ethnicity, education, country of birth, marital status and income). At the time of data analyses, 79 participants were excluded because of missing

questionnaire data, leaving 137 cases and 158 controls for a total of 295 participants.

In both case and control groups, standardized genital exams of the infants were conducted by pediatricians soon after birth to obtain information on testicular position (descended vs. undescended), with verification of cryptorchidism by pediatric urologists. Testicles were initially defined as nonpalpable or palpable. Nonpalpable testes were further classified as vanishing, abdominal, or atrophic at the time of surgery. Palpable testes were considered to be inguinal or prescrotal. Ectopic testicles were defined to have a perineal, femoral, prepubic, contralateral scrotal, or superficial inguinal pouch location. The superficial inguinal pouch location was only identified at the time of surgery because this cannot be distinguished from an inguinal testis on physical exam alone. For all cryptorchid children, a chart review was carried out post-surgery by a urologist and the research coordinator to obtain details of the precise location of the testes.

### Hair Sample Collection

Hair was used as a matrix to assess the PBDE exposure of mothers and children because previous studies reported a positive correlation between serum and hair PBDE concentrations, especially for tetra- to hexa BDE congeners (Poon et al. 2014; Zheng et al. 2014). Sufficient samples of hair for the PBDE assay were collected at the time of recruitment from all mothers and approximately a third of the babies (many babies had too little or no hair): maternal–child paired samples were collected for 57 cases and 50 controls. Using stainless steel scissors, 50–100 mg of hair was collected from the mothers and as much hair as possible from the babies: the hair was cut within 1 cm from the scalp at the posterior vertex (Aleksa et al. 2012). The hair samples were stored in sealed envelopes in the dark at  $4^{\circ}\text{C}$  until assayed at the Hospital for Sick Children.

### Hair Sample Analyses

To standardize the hair analyses, PBDEs were measured in the first 3–4 cm of hair closest to the root. The methodology for adult and child hair PBDE measurements was established previously (Aleksa et al. 2012; Carnevale et al. 2014; Poon et al. 2014). In brief, samples were rinsed with Milli-Q water and dried with paper towels to remove dust from the hair surface (Poon et al. 2015). The hair was then weighed (5–30 mg) and finely cut into 1–2 mm pieces. Samples were analyzed by GC-MS for eight PBDE congeners: BDE-28, -47, -99, -100, -153, -154, -183, and -209. Quantification was performed using five-point calibration curves whereby standards (Wellington Laboratories) were added to extracts of a single pool of “blank” hair because it contained negligible levels of the eight PBDEs being measured. The peak area ratios of congeners BDE-28 to -183 to their internal standard (F-BDE-69) and BDE-209 to its standard ( $^{13}\text{C}_{12}$ -BDE-209) were calculated. The area ratios in blank hair were subtracted from the sample area ratios prior to plotting against the calibration curve to quantify the PBDEs. The PBDE levels were corrected for dry weight of each sample. The limits of detection (LOD) ranged from 1 to 4 pg/mg and the limits of quantification (LOQ) from 3 to 12 pg/mg. The percent recoveries ranged from 100% to 120% with the exception of BDE-47 (135%), and the percent CVs ranged from 13% to 19% with the exception of BDE-209 (33%). Machine-read values were used for concentrations that fell between the LOD and LOQ. Values below the LOD were imputed using multiple imputation (see below). Analyses were limited to those congeners with quantification frequencies  $>50\%$  (BDE-28, -47, -99, -100, -153, -154, and -209) and their sum.

With a quantification frequency of 40.2%, BDE-183 was not considered in statistical analyses.

### Statistical Analyses

PBDE concentrations were heavily right-skewed and were thus log<sub>10</sub>-transformed to reduce the influence of outliers. We used Pearson's correlations and analysis of variance (ANOVA) to estimate bivariate associations. Multivariable associations between maternal hair PBDE concentrations and cryptorchidism case status were estimated using multiple logistic regression. Because the proportional odds assumption did not hold for all analyses ( $p < 0.05$ ), we used multinomial (rather than ordinal) logistic regression to evaluate associations with cryptorchidism severity based on testis position (i.e., inguinal, ectopic, intra-abdominal) and the number of testes affected (i.e., unilateral, bilateral). Potential confounders were identified based on directed acyclic graphs (DAGs) and included maternal age (continuous), birthplace, ethnicity, marital status, income, education, body mass index (continuous), alcohol and caffeine consumption during pregnancy (yes vs. no), smoking during pregnancy, gestational diabetes, use of assisted reproductive techniques, child age at examination, and family history of cryptorchidism (as shown in Table 1 and below).

Final models included variables that were loosely associated with the outcome ( $p < 0.20$ ) in bivariate analyses (i.e., maternal age, birthplace, ethnicity, marital status, income, highest level of education, and paternal history of cryptorchidism). Missing values were imputed based on multiple imputation by chained equations (MICE) using predictive mean matching for missing covariates and interval-censored regression for PBDE values below the LOD (van Buuren et al. 1999). MICE can use a variety of prediction models that may include variables of any form and with varying levels of missingness to impute missing values. Multiple imputation has been shown to generate valid parameter estimates and, as opposed to single substitution, properly estimates variance by taking into account the uncertainty associated with imputed values (Lubin et al. 2004; Rubin 1976, 1987). Estimates and their variance were estimated by generating 50 imputations and using Rubin's formula (Rubin 1976, 1987). All analyses were conducted using Intercooled STATA version 13.1 (StataCorp).

## Results

### Participant Characteristics

The mean maternal age at the time of interview was 33 years (range, 18–48). As shown in Table 1, the majority of the mothers were born in North America (73%) with 71% born in Canada, Caucasian (72%), married or living as married (96%), and with a household income  $\geq 60,000$ /year (68%). Relative to controls, cases were less likely to be Caucasian, were younger, had a lower family income, and were more likely to have a paternal and family history of cryptorchidism. There were no differences between the geometric mean total hair PBDE levels by demographic characteristics or family history of urogenital anomalies.

### Hair PBDE Levels

The geometric means and distribution of the maternal PBDE hair concentrations and their sums for the cases and controls are provided in Tables 2 and 3. Except for BDE-209, PBDE congeners were moderately intercorrelated ( $r = 0.36 - 0.71$ ;  $p < 0.001$ ; see Table S1). BDE-47 and -209 had the highest levels, followed by BDE-99 and -100, in both cases and controls. The geometric means of the individual PBDEs was significantly higher in the

case mothers than the controls for BDE-99 ( $p < 0.002$ ), BDE-100 ( $p < 0.001$ ), and BDE-154 ( $p < 0.04$ ). Tables S2 and S3 provide data for the case and control infants. Again, BDE-47 and -209 were the highest in both groups, followed by BDE-99 and -100. PBDE concentrations in paired maternal and infant hair samples were moderately correlated among both the cases and controls ( $r = 0.34 - 0.71$ ;  $p < 0.01 - 0.001$ ) (see Table S4); cases also showed a moderate correlation for the  $\sum$  PBDEs ( $r = 0.41$ ;  $p < 0.001$ ). We found no association between hair PBDE concentrations and child age at examination, breastfeeding status or duration, or hair coloring (data not shown).

### Association between Hair PBDE Levels and Cryptorchidism

Figure 1 presents associations between individual BDEs as well as total PBDE levels in maternal hair and the odds of cryptorchidism. Every 10-fold increase in maternal hair BDE-99 [OR = 2.53 (95% CI: 1.29, 4.95;  $p < 0.007$ )], BDE-100 [OR = 2.45 (95% CI: 1.31, 4.56;  $p < 0.005$ )] or BDE-154 [OR = 1.88 (95% CI: 1.08, 3.28;  $p < 0.026$ )] was associated with elevated risk of cryptorchidism in male infants.

### Multinomial Logistic Regression Model

Data on the number of affected testes and site of the testis for all cryptorchid children (as well as the related geometric means of maternal hair PBDEs) are presented in Table S5. Multinomial logistic regression models confirmed an association of BDE-99, -100, and -154 with inguinal localization of the testes (Table 4). Statistical power to detect associations with ectopic ( $n = 7$ ) or intra-abdominal ( $n = 19$ ) cryptorchidism was limited due to the small number of cases. A similar lack of power was observed when associations of PBDEs were evaluated based on whether cryptorchidism was unilateral (one testis undescended;  $n = 108$ ) versus bilateral (both testes undescended;  $n = 17$ ); significant associations were observed only with the unilateral cases (data not shown).

## Discussion

We report here a significant association between maternal exposure to BDE-99, -100, and -154 and elevated risks of cryptorchidism in male infants. *In vivo* and *in vitro* studies of these congeners have demonstrated potent anti-androgenic properties of BDE-100, similar to those of the classical antiandrogen, flutamide, and a 10- to 80-fold lower effect of BDE-99 (Hamers et al. 2006; Harju et al. 2007; Kojima et al. 2009; Lilienthal et al. 2006; Stoker et al. 2005; Yang et al. 2009). One published study that included BDE-154 suggested that it has weak anti-androgenic activity (Stoker et al. 2005). Although BDE-100 has also been predicted to have weak estrogenic activities (Kojima et al. 2009; Meerts et al. 2001; Papa et al. 2010; Yang et al. 2009), these activities are several orders of magnitude lower than observed with estradiol. Overall, these data suggest that the anti-androgenic properties of BDE-99, -100, and -154 may help to explain their association with the disruption of testicular descent in our study.

BDE-47 and -209 were also frequently detected in the hair samples. BDE-47 has been shown to have significant anti-androgenic activities ( $\sim 5$ - to 10-fold less than BDE-100 but higher than BDE-99) in both *in vivo* and *in vitro* assays, whereas BDE-209 has little to no activity (Hamers et al. 2006; Stoker et al. 2005). The lack of an association between BDE-47 and risk of cryptorchidism was surprising given our proposed mechanism. This suggests that PBDEs may impact cryptorchidism by additional mechanisms of action. One possibility is that PBDEs are actively metabolized in human tissues leading to the formation of

**Table 1.** Geometric means and standard deviations of maternal hair  $\Sigma$ PBDE<sup>a</sup> concentrations by demographic characteristics and family history of urogenital anomalies among cryptorchidism cases ( $n = 137$ ) and controls ( $n = 158$ ).

	Cases		Controls	
	No. (%)	GM <sup>b</sup> (GSD <sup>c</sup> )	No. (%)	GM (GSD)
Birthplace				
North America	92 (67)	50.3 (1.9)	124 (78)	43.6 (2.0)
South America	6 (4)	55.0 (2.0)	5 (3)	79.5 (1.7)
Europe	12 (9)	48.0 (2.0)	13 (8)	55.1 (2.0)
Other	27 (20)	52.7 (1.9)	16 (10)	43.9 (1.9)
Ethnic background				
Caucasian	90 (69)	46.2 (1.9)	121 (81)	45.5 (2.0)*
Asian	8 (6)	48.4 (1.8)	10 (7)	41.2 (1.6)
Hispanic	12 (9)	52.5 (2.1)	5 (3)	72.1 (1.5)
Arab	9 (7)	68.0 (1.5)	3 (2)	32.3 (2.2)
Other	12 (9)	68.7 (1.8)	11 (7)	46.5 (2.2)
Marital status				
Married/living as married	128 (94)	50.0 (1.9)	154 (97)	45.1 (2.0)
Single (never married)	6 (4)	42.4 (1.9)	1 (1)	31.9 (0.0)
Other	2 (1)	100.4 (1.0)	3 (2)	66.2 (1.2)
Household income (Canadian dollars)				
0–29,999	10 (9)	59.8 (2.0)	4 (3)	50.2 (1.5)***
30,000–59,999	20 (18)	54.1 (1.8)	9 (7)	45.8 (2.3)
60,000–89,999	34 (30)	46.0 (1.9)	31 (20)	40.1 (1.9)
≥ 90,000	49 (43)	45.0 (2.0)	88 (67)	47.8 (1.9)
Highest education level				
Less than high school	63 (47)	53.4 (2.0)	37 (26)	37.1 (2.2)***
High school	41 (30)	49.8 (1.8)	62 (43)	45.7 (1.9)
More than high school	31 (23)	44.5 (1.8)	46 (32)	49.4 (2.0)
Drank during pregnancy				
Yes	10 (9)	49.3 (1.9)	12 (8)	44.4 (1.6)
No	104 (91)	48.8 (2.0)	143 (92)	45.3 (2.0)
Smoked during pregnancy				
Yes	3 (5)	73.2 (1.3)	4 (3)	45.3 (1.8)
No	58 (95)	47.8 (1.9)	154 (97)	45.3 (2.0)
Mother's age (years)				
<25	7 (5)	66.7 (2.0)	1 (1)	11.8 (0.0)**
25–29	30 (23)	47.8 (1.8)	23 (15)	46.5 (1.9)
30–34	46 (35)	49.4 (2.1)	74 (47)	47.3 (2.1)
35–39	41 (32)	49.0 (1.8)	44 (28)	41.2 (1.9)
≥ 40	6 (5)	48.5 (1.6)	14 (9)	49.7 (1.7)
BMI (kg/m <sup>2</sup> )				
<20	13 (11)	45.4 (1.8)	19 (13)	45.6 (2.3)
20–24.9	61 (50)	49.1 (2.0)	74 (51)	50.8 (1.8)
25–29.9	32 (26)	57.3 (1.9)	38 (26)	39.2 (1.9)
30–34.9	13 (11)	39.2 (2.1)	11 (8)	47.0 (2.4)
≥ 35	4 (3)	58.2 (1.5)	3 (2)	32.4 (1.5)
Schooling (years)				
<15	22 (29)	39.9 (2.1)	18 (16)	54.5 (2.3)
15–19	40 (53)	45.0 (1.8)	77 (68)	44.2 (1.9)
≥ 20	14 (18)	46.0 (1.9)	19 (17)	59.6 (2.1)
Dependents (no.)				
2	13 (11)	67.0 (2.1)	4 (3)	31.3 (2.0)
3	52 (42)	47.0 (2.1)	67 (49)	45.0 (2.2)
4	38 (31)	46.8 (1.8)	48 (35)	42.2 (1.8)
5	16 (13)	53.9 (1.8)	11 (8)	49.2 (2.2)
≥ 6	4 (3)	51.8 (1.8)	7 (5)	47.9 (1.6)
Child's age (months)				
3–7.9	47 (34)	51.0 (1.8)	64 (42)	45.0 (1.9)
8–12.9	53 (39)	52.4 (1.9)	65 (42)	47.9 (2.0)
13–18	37 (27)	46.6 (2.0)	25 (16)	37.3 (2.0)
Paternal history of cryptorchidism/hypospadias				
None	113 (90)	50.2 (1.9)	126 (98)	45.6 (2.0)*
Cryptorchidism	11 (9)	51.9 (1.8)	2 (2)	80.7 (3.4)
Hypospadias	1 (1)	67.2 (0.0)	0 (0)	0.0 (0.0)
Family history of cryptorchidism				
Yes	18 (13)	62.3 (1.9)	5 (4)	32.7 (2.6)**
No	119 (87)	48.7 (1.9)	132 (96)	46.0 (2.0)
Family history of hypospadias				
Yes	1 (1)	48.1 (0.0)	0 (0)	0.0 (0.0)
No	136 (99)	50.3 (1.9)	136 (100)	45.8 (2.0)

(Continued)

Table 1. Continued

	Cases		Controls	
	No. (%)	GM <sup>b</sup> (GSD <sup>c</sup> )	No. (%)	GM (GSD)
Use of assisted reproductive techniques				
Ovulation induction	3 (2)	43.2 (1.9)	4 (3)	51.2 (1.9)
Artificial insemination	0 (0)	0.0 (0.0)	3 (2)	34.7 (1.9)
<i>In vitro</i> fertilization	6 (4)	49.2 (1.3)	6 (4)	43.8 (2.8)
None	126 (93)	51.0 (1.9)	135 (91)	45.9 (1.9)
Gestational diabetes				
Yes	13 (10)	60.7 (1.5)	11 (7)	40.2 (2.1)
No	122 (90)	49.7 (1.9)	147 (93)	45.8 (2.0)

<sup>a</sup>Summed congeners include BDE-28, -47, -99, -100, -153, -154, and -209.

<sup>b</sup>Geometric mean.

<sup>c</sup>Geometric standard deviation. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001 based on chi-squared tests comparing frequency distributions between total cases and controls. ΣPBDE did not significantly differ across demographic characteristics.

hydroxylated and methoxylated BDEs; some of these metabolites may be more bioactive than the parent compounds (Hamers et al. 2006; Hamers et al. 2008; Kojima et al. 2009; Meerts et al. 2001; Yang et al. 2011). The fact that we measured the parent compounds rather than their metabolites may explain in part why we observed associations with some congeners but not others.

One limitation of our study is the inability to rule out the possible influence of alternative BFRs that may be co-eluting with the PBDEs during the GC-MS analysis. For example, BDE-99 elutes very close to 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), a component of Firemaster 550 (Fan et al. 2016; Liu et al. 2015; Stapleton et al. 2008). In addition, BDE-154 co-elutes with 2,2',4,4',5,5'-hexabromobiphenyl (BB-153), a major component in previous commercial mixtures of polybrominated biphenyls (Korytár et al. 2005). Given that we were using the same source of hair for the calibration curves, it is possible that, although this hair sample did not contain EH-TBB or BB-153, that other hair samples did.

Two possible mechanisms may underlie the association between exposure to specific PBDEs and cryptorchidism: *a*) competition at the androgen receptor level, to block the effects of endogenous androgens (e.g., in the gubernaculum muscle or inguinoscrotal fat pad) (Barthold et al. 2016; Kaftanovskaya et al. 2012) and/or *b*) effects on the Leydig cell in the fetal testes, resulting in endogenous androgen insufficiency (Houk et al. 2004; Wang et al. 2009). Stoker et al. (Stoker et al. 2005) reported that BDE-99 and -100 display antagonistic properties in the rat ventral prostate binding assay (IC<sub>50</sub>s of 33 and 3 μM, respectively), while BDE-154 has a weak effect; they also found that BDE-100 is a true competitor at the androgen receptor level

(K<sub>i</sub> = 1 μM) and inhibits dihydrotestosterone-induced androgen receptor activation in a concentration-dependent manner. Similar data for BDE-99 and -100 have been obtained by Hamers et al. (2006) using an *in vitro* androgen receptor CALUX assay. These findings indicate that BDE-100 is a potent competitive inhibitor of the androgen receptor and that the other two BDEs also have antiandrogen receptor properties. Direct effects of these PBDE congeners on the human fetal testes have not been reported. Thus, whether these congeners have effects on endogenous human androgen production is still unknown.

Cryptorchidism studies in animal models have clearly implicated roles for both specific genes (*Insl-3*, *Rxfp2*) and the fetal hormonal (androgenic) environment in the migration of testes (Barthold 2008; Gorlov et al. 2002; Huang et al. 2012; Nef and Parada 1999; Virtanen and Toppari 2008). However, similar evidence in the human male is limited despite the fact that cryptorchidism is a relatively common finding in normal term newborn males. Only a few of these infants have been shown to have *INSL-3* or *RXFP2* gene mutations; in the rare newborns with complete androgen insensitivity due to absence of a functional androgen receptor, the testes remain in the inguinal or groin area (Bay et al. 2011; Feng et al. 2009; Ferlin et al. 2009). Failure to identify genome-wide significant markers associated with nonsyndromic cryptorchidism has led to the recent suggestion that cryptorchidism is the result of a complex multilocus genetic susceptibility with the potential for additional risk from *in utero* environmental exposures (Barthold et al. 2015).

It is well accepted that the intrauterine environment plays a critical role in fetal development in general and there is increasing evidence that this is true for cryptorchidism as well. A

Table 2. Detection frequencies, geometric means and percentiles of maternal hair PBDE concentrations among cases (*n* = 137).

PBDEs	LOD <sup>a</sup> , LOQ <sup>b</sup> (pg/mg)	Detect freq <sup>c</sup> (%)	Quant freq <sup>d</sup> (%)	GM <sup>e</sup> (pg/mg)	95% CI <sup>f</sup> (pg/mg)	Min (pg/mg)	25th percentile (pg/mg)	Median (pg/mg)	75th percentile (pg/mg)	Max (pg/mg)
Σ PBDEs <sup>g</sup>				50.27	45.12, 56.01	8.92	34.27	53.03	85.99	167.42
BDE-28	1.00, 4.00	74.45	24.09	2.13	1.84, 2.47	<LOD	<LOD	2.08	3.77	17.98
BDE-47	3.00, 8.00	85.40	58.39	8.89	7.69, 10.28	<LOD	4.70	10.01	17.03	75.40
BDE-99	2.00, 7.00	86.13	56.20	7.14	6.14, 8.31	<LOD	4.09	7.94	12.71	50.10
BDE-100	1.00, 4.00	89.78	69.34	6.12	5.11, 7.32	<LOD	3.28	7.40	13.47	46.30
BDE-153	2.00, 5.00	71.53	34.31	3.63	3.17, 4.17	<LOD	<LOD	3.50	7.06	21.65
BDE-154	1.00, 4.00	92.70	50.36	4.08	3.49, 4.76	<LOD	2.16	4.03	8.00	27.08
BDE-183	4.00, 12.00	40.15	5.11	4.32	3.92, 4.76	<LOD	<LOD	<LOD	7.38	24.79
BDE-209	1.00, 3.00	93.43	86.86	9.08	7.64, 10.80	<LOD	5.62	11.37	16.67	78.30

<sup>a</sup>Limit of detection.

<sup>b</sup>Limit of quantification.

<sup>c</sup>Detection frequency.

<sup>d</sup>Quantification frequency.

<sup>e</sup>Geometric mean.

<sup>f</sup>95% confidence interval.

<sup>g</sup>Summed congeners include BDE-28, -47, -99, -100, -153, -154, and -209.

**Table 3.** Detection frequencies, geometric means and percentiles of maternal hair PBDE concentrations among controls ( $n = 158$ ).

PBDEs	LOD <sup>a</sup> , LOQ <sup>b</sup> (pg/mg)	Detect freq <sup>c</sup> (%)	Quant freq <sup>d</sup> (%)	GM <sup>e</sup> (pg/mg)	95% CI <sup>f</sup> (pg/mg)	Min (pg/mg)	25th percentile (pg/mg)	Median (pg/mg)	75th percentile (pg/mg)	Max (pg/mg)
∑ PBDEs <sup>g</sup>				45.35	40.77, 50.43	8.57	30.76	44.48	74.39	247.52
BDE-28	1.00, 4.00	70.89	31.65	2.42	2.09, 2.81	<LOD	<LOD	2.76	4.64	14.46
BDE-47	3.00, 8.00	85.44	60.76	9.03	9.03, 10.26	<LOD	6.07	9.58	15.35	72.70
BDE-99	2.00, 7.00	79.11	37.97	5.11	4.46, 5.86	<LOD	2.66	5.97	8.71	79.40
BDE-100	1.00, 4.00	91.14	49.37	4.09	3.56, 4.70	<LOD	2.33	3.99	7.45	57.97
BDE-153	2.00, 5.00	68.99	23.42	3.10	2.77, 3.47	<LOD	<LOD	2.83	4.90	30.20
BDE-154	1.00, 4.00	76.58	46.84	3.28	2.76, 3.90	<LOD	1.08	3.57	6.49	42.59
BDE-183	4.00, 12.00	52.53	14.56	5.20	4.66, 5.79	<LOD	<LOD	4.14	9.11	32.06
BDE-209	1.00, 3.00	83.54	74.05	7.07	5.67, 8.81	<LOD	2.77	8.45	18.19	226.65

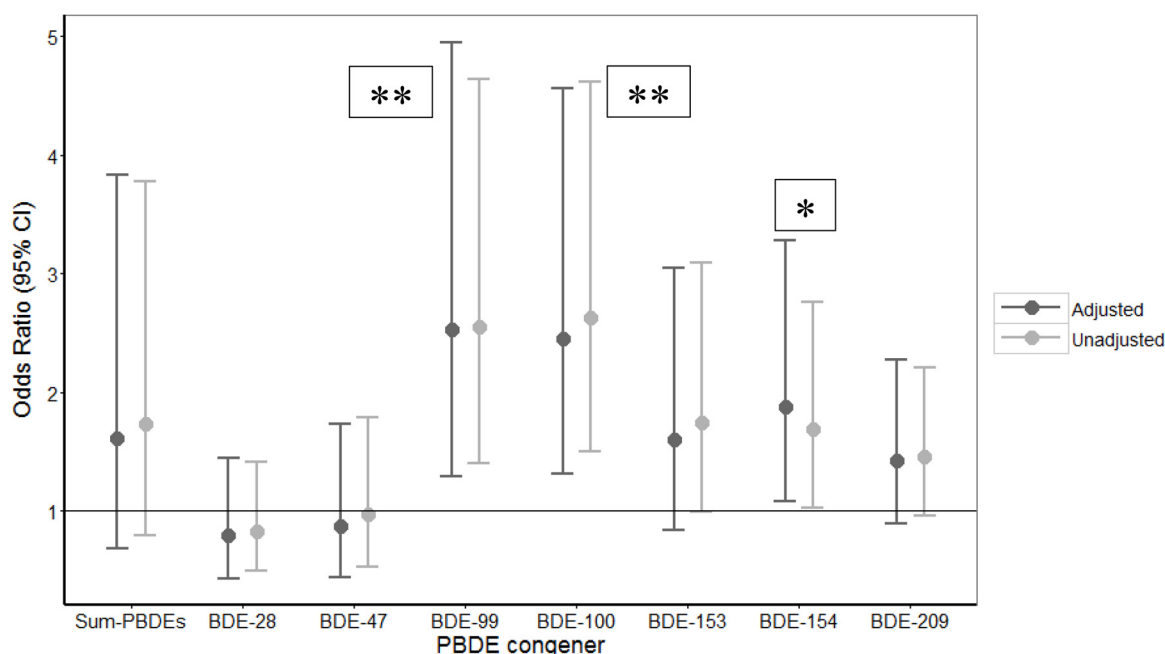
<sup>a</sup>Limit of detection.<sup>b</sup>Limit of quantification.<sup>c</sup>Detection frequency.<sup>d</sup>Quantification frequency.<sup>e</sup>Geometric mean.<sup>f</sup>95% confidence interval.<sup>g</sup>Summed congeners include BDE-28, -47, -99, -100, -153, -154, and -209.

number of epidemiological studies have found significant associations between an increased risk of cryptorchidism and prematurity, low birth weight and maternal gestational diabetes; the roles of smoking, alcohol consumption, acetaminophen use, maternal BMI, and assisted reproduction techniques remain controversial (Zhang et al. 2015). There have also been studies suggesting a specific role for environmental chemicals that readily cross the placenta and have endocrine disruptor properties (e.g., PBDEs, phthalates, tributyltin, bisphenol A, pesticides, perfluorinated compounds, dioxins) but these have been limited to one or two papers per chemical (Agopian et al. 2013; Bay and Anand-Ivell 2014; Christen et al. 2014; Doucet et al. 2009; Main et al. 2007; Rouiller-Fabre et al. 2015; Virtanen and Adamsson 2012).

In general, studies have focused on the anti-androgenic and/or pro-estrogenic properties of endocrine disrupting chemicals because the androgenic *in utero* environment is crucial in the testicular migration process (Christen et al. 2014; Dean and Sharpe 2013; Jain and Singal 2013; Thankamony et al. 2014). Additional evidence comes from the measurement of anogenital distance

(AGD) in humans. AGD is well-known to be related to prenatal hormonal exposure and has been positively correlated with testis size, sperm count, and testosterone levels (Dean and Sharpe 2013; Swan et al. 2005). Mean values of AGD in infant males with cryptorchidism are significantly shorter than in healthy boys, suggesting that global inhibition of androgen production and/or action plays a role in the pathogenesis (Jain and Singal 2013; Thankamony et al. 2014).

We had hypothesized that the severity of the cryptorchidism (abdominal location vs. inguinal/ectopic or unilateral vs. bilateral) might be associated with maternal PBDE exposure. Unfortunately, the numbers of abdominal and bilateral cases were too low to derive a definitive conclusion. Despite this, our study has three major strengths. First, because cryptorchidism can spontaneously resolve in the first 6 months of life, we only considered as cases those infants who were confirmed at the time of orchidopexy; thus, we analyzed an unambiguous case cohort. This population differs from the cases described in the cohort of Danish mothers and infants where Main et al. (Main et al. 2007)

**Figure 1.** Unadjusted and adjusted association between maternal hair PBDE concentrations and odds of cryptorchidism. \* $p < 0.05$ , \*\* $p < 0.01$ . Error bars represent 95% confidence intervals. Adjusted models included maternal birthplace, ethnicity, marital status, income, age, education, and paternal history of cryptorchidism.

**Table 4.** Association between maternal hair PBDE concentrations and testis location ( $n = 295$ ).

PBDEs	Testis location	OR <sup>a</sup> (95% CI) <sup>b</sup>
Σ PBDEs <sup>c</sup>	Ectopic	3.83 (0.24, 61.23)
	Inguinal	1.60 (0.62, 4.09)
	Intra-abdominal	1.32 (0.24, 7.33)
BDE-28	Ectopic	0.75 (0.13, 4.23)
	Inguinal	0.86 (0.45, 1.64)
	Intra-abdominal	0.59 (0.18, 1.87)
BDE-47	Ectopic	1.23 (0.14, 10.68)
	Inguinal	0.86 (0.41, 1.80)
	Intra-abdominal	0.83 (0.22, 3.14)
BDE-99	Ectopic	5.04 (0.48, 53.47)
	Inguinal	2.45 (1.19, 5.04)*
	Intra-abdominal	2.64 (0.70, 9.98)
BDE-100	Ectopic	8.37 (0.87, 80.35)
	Inguinal	2.42 (1.23, 4.75)**
	Intra-abdominal	1.97 (0.57, 6.74)
BDE-153	Ectopic	1.84 (0.26, 12.89)
	Inguinal	1.88 (0.93, 3.80)
	Intra-abdominal	0.79 (0.23, 2.79)
BDE-154	Ectopic	1.36 (0.27, 6.97)
	Inguinal	1.98 (1.08, 3.62)*
	Intra-abdominal	1.75 (0.58, 5.32)
BDE-209	Ectopic	2.11 (0.49, 9.14)
	Inguinal	1.35 (0.82, 2.25)
	Intra-abdominal	1.63 (0.61, 4.33)

Note: Models adjusted for maternal birthplace, ethnicity, marital status, income, age, education, and paternal history of cryptorchidism.

<sup>a</sup>Odds ratio.

<sup>b</sup>95% confidence intervals.

<sup>c</sup>Summed congeners include BDE-28, -47, -99, -100, -153, -154, and -209. \* $p < 0.05$ , \*\* $p < 0.01$ .

found a significant association between the levels of several PBDEs in maternal breast milk (1–3 months postnatal) and cryptorchidism at birth: of  $n = 29$  cases, only 4 remained cryptorchid at 3 months. Thus, the Danish study was primarily focused on an association between maternal PBDE exposure and delayed migration of the infant testes. Second, we used multiple imputation to impute PBDE values below the level of detection. This method allowed us to take advantage of the intercorrelation between congeners as well as the predictive power of other covariates to impute undetected PBDE values while accounting for the uncertainty of these values in variance estimates. Finally, we collected data on a large number of potential confounders.

The major advantage of using hair as a biomarker of environmental exposure is that it can be collected in a relatively noninvasive fashion. We have assumed that the PBDE levels in 3–18 months postnatal maternal hair samples are reflective of exposure during the gestational period. This assumption is based on three strong pieces of evidence: *a*) the lack of an association between maternal hair PBDEs in this study and child age at the time of sample collection or breastfeeding duration; *b*) the stability of PBDEs over months to years in serum (Castorina et al. 2011; Imm et al. 2009; Makey et al. 2014); and *c*) the long half-lives of the PBDEs, especially the penta- and hexa-BDEs (1–7 years) that we have found to be associated with risk of cryptorchidism (Thuresson et al. 2006; Trudel et al. 2011).

Unless treated by surgery very early in childhood, consequences of cryptorchidism may include subfertility and testicular cancer in adulthood (Kollin and Ritzén 2014). Recently, a European Union expert panel not only identified “strong toxicological evidence for cryptorchidism due to prenatal PBDE exposure,” but also estimated an annual cost-of-illness at €117–130 million (Hauser et al. 2015). Thus, there are both reproductive health and economic reasons to decrease the present occurrence rate of cryptorchidism, possibly by decreasing

maternal exposure to specific environmental chemicals, such as PBDEs, prior to and during gestation.

## Conclusions

Our results suggest an association between maternal exposure to BDE-99, -100, and -154, as measured in maternal hair, and abnormal migration of testes in the male fetus; this may be due to the anti-androgenic properties of these PBDEs, especially BDE-100.

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